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Effect of Sidr honey on production of bacteriocin like inhibitory substances in some pathogenic bacteria

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ABSTRACT

Understanding the link between bacterial growth rate and bacteriocin production is necessary to achieve the highest output. The study aimed to determine the effect of sidr honey on stimulating the formation and release of bacteriocin-like inhibitory substances in pathogenic bacteria. Forty-eight isolates were obtained from different sources, and one isolate of each species (*Pseudomonas aeroginoa, Proteus mirabilis, Proteus vulgaris, Staphylococcus aureus, Staphylococcus haemolyticus, Enterobacter cloacae, Salmonella enteric, Escherichia coli. Klebsiella Pneumonaie*) were selected to conduct the study. The findings revealed significant differences in the production of BLIS in all isolates, as well as in the growth of bacteria, where there was an increase in both bacterial density and output of BLIS when using a culture medium supplemented with 1% Sidr honey, except for *P. mirabilis* and *Enterobacter cloacae*. Intestinal *Salmonella and Klebsiella pneumonia*, where the density of bacteria decreases when adding 1% of Sidr honey. the addition of sidr honey stimulated the production of BLIS in P. mirabilis. It increased the activity in all isolates, but the density of non-BLIS producers decreased compared to culture media without sidr honey.

Keywords: Bacteriocin like inhibitory substances, Sidr honey, Pathogenic bacteria.
Article type: Research Article.

INTRODUCTION

Bacteriocins are antimicrobial peptides produced by bacteria and play an important role in their competition for food and ecological niche in the microbiota (Yang *et al.* 2014). These peptides are highly effective against certain bacteria, including multidrug-resistant (MDR) strains, while producer strains remain resistant to the effect of peptides (Meade, Slattery & Garvey 2020). Due to the excessive misuse of antibiotics, drug-resistant bacteria have become one of the major problems around the world, which can affect both humans and animals, so finding alternatives to avoid the problem of drug resistance has become an urgent necessity(Zhi *et al.* 2018) .Bacteriocins represent one of the potential alternatives to get rid of diseases caused by antibiotic- resistant bacteria(Soltani *et al.* 2021). Bacteriocins possess valuable unique traits such as pH and thermal stability at wide ranges, and there has been no research of bacteriocin- resistant bacteria, perhaps because of their rapid mechanisms of acting that prevent bacteria from developing resistance even at low concentrations(Perez, Zendo & Sonomoto 2014). In addition to the great importance of bacteriocins in eliminating MDR bacteria, bacteriocins have various applications, such as food preservative (Verma *et al.* 2022), anticancer (Drago 2019),aquaculture(Nayak *et al.* 2022), antifungal .(Mohsin 2021). Understanding the link between bacterial growth rate and bacteriocin production is necessary to achieve the highest output. Many studies have shown that the synthesis of bacteriocins

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occurs during the exponential growth stage and is influenced by a wide range of variables, including temperature, pH, aeration, incubation time, and the type and concentration of nutrients in the culture medium (Yang *et al.* 2018; Al-Taie, Al-Musawi & Rasheed 2022). Honeybees produce honey by sucking on the secretions of flowers or eating the nectar and blossoms of flowers. The source of the plants that the bees eat determines how honey is composed (Eteraf-Oskouei & Najafi 2013). However, honey of any origin is primarily composed of carbohydrates. The principal carbohydrates contained in honey are glucose, sucrose, and fructose, which account for 95% of the dry matter and are responsible for their nutritional value.(Hegazi *et al.* 2022).Also honey contains a wide range of nutritional and bioactive qualities owing to its high content of proteins, amino acids, minerals, flavonoids, enzymes, organic acids, and phenolic acids (Adaškevičiūtė *et al.* 2019; Djebli *et al.* 2021). Previous research focused on the production of bacteriocin from lactic acid bacteria. Hence, the current study aims to investigate the production of bacteriocin-like inhibitory substances using pathogenic bacteria and the effect of Sidr honey on bacterial growth and BLIS production.

MATERIALS AND METHODS

Collection of isolates

The present study was conducted on 48 bacterial isolates from different clinical sources of Shaikh Zayed Hospital in Baghdad. The isolates were *(P. aeroginoa, P. mirabilis, P. vulgaris, S. aureus, S. haemolyticus, En. cloacae, S. enteric, E. coli. K. Pneumonia);* these isolates were diagnostic by biochemical tests and then by Vitek 2 system. This isolates does not include any animal experiments or human studies.

Screening of BLIS production in pathogenic bacteria

BLIS producing isolates were screened by the Agar Well Diffusion (AWD) method as follows: Tubes contained ten ml of Brain Heart Infusion Broth (BHIB) were added with 2 McFarland standards (3×10^8 CFU/ml) of the producer isolate. After that, the tubes were incubated at 37 °C for 24 hour. the cultured broth were centrifuged at 6000 rpm for 10 minutes after incubation, and the cell-free supernatant (CFS) was obtained to study its inhibitory activity (Hashim *et al.* 2017).

Determining the effect of Sidr honey on BLIS production and bacterial growth

Sidr honey was prepared to 1% concentration by sequentially adding 1 ml to 99 ml of culture broth medium to evaluate the impact of Sidr honey on BLIS production and bacterial growth. The spectrophotometer measured Bacterial density with a wavelength of 600 nm (A_{600}) for the initial inoculum and after incubation for 24 h for the control (culture without Sidr honey) and the culture supplemented with Sidr honey (1%)(Wood, Osman and Wade, 2019).

2.4 Antimicrobial activity of BLIS

Antimicrobial activity of BLIS was measured by the AWD method against vancomycin-resistant *E. faecalis, E. coli, Salmonella enterica, pseudomonas aeroginosa, Staphylococcus aureus, and Klebsiella pneumonia* as indicator strains. The CFS of each isolate was assayed for the presence of BLIS as follows: A Mueller-Hinton agar plate was cultured with 0.1 ml of the indicator bacteria (1.5 x 108 CFU/ml), and wells of 5 mm in diameter were cut by using a cork borer. Following, 0.1 ml of CFS was put in the wells, and after 18 hours of incubation, the diameter of the inhibitory zone was measured**.**

Statistical Analysis:

The Statistical Analysis System- SAS (2012) application was utilized to determine the effect of various variables in study parameters. In this research, the T-test was employed to compare statistically significant means (Cary, 2012).

RESULTS

Screening of BLIS production in pathogenic bacteria

From 48 isolates, 26 BLIS-producing isolates were obtained, as shown in Table 1. The results show that *Staphylococcus* has the highest production of BLIS compared to other species.

Effect of Sidr honey on BLIS production and bacterial growth

The inhibitory activity of BLIS against indicator isolates and optical density of bacteria shown in table 2. There is increase in BLIS production and Significant differences in between the medium with and without 1% sidr honey

as well as induced its production in *P. mirabilis*. The results also shows that the growth rate increases in *S. aureus, S. haemolyicus, P. aeroginosa*, and E. coli when 1% sidr honey added to culture medium compared to the culture medium without sidr honey except in *P. mirabilis, S. enterica, P. vulgaris, and En. cloacae*, the density of bacteria decreased after incubation for 24h at an initial concentration of 0.2 Fig. 1.

Table 1. Numbers and percentages of BLIS and non-BLIS production of bacterial isolates.

Bacterial species	No. of BLIS production (%)	No. of non BLIS production (%)
S. aureus 19	15/19(79)	4/19(21)
S. haemolyticus 2	2/2(100)	0/2(0)
E. coli 12	6/12(50)	6/12(50)
S. enterica 1	1/1(100)	0/1(0)
P. aeruginosa 8	3/8(37)	5/8(63)
E. cloacae 1	0/1(0)	1/1(100)
P. mirabilis 2	0/2(0)	2/2(100)
P. vulgaris 1	0/1(0)	1/1(100)
K. pneumoniae 2	0/2(0)	2/2(100)

Table 2. Comparison between control and 1% sider honey in inhibition zone and OD of bacteria concentration with difference species.

Species	initial con.	Inhibition zone (mm)			OD of bacterial concentration		
		Control	1% sider honey	T-test	Control	1% sider honey	T-test
S. aureus	0.2	8.10	33.13	5.39 **	0.820	1.672	0.692 *
S. haemolyticus		7.0	28.03	6.22 **	1.24	2.19	0.651 *
E. coli		13.20	17.20	3.07 NS	1.4	2.05	0.548 *
S. enterica		15.33	17.13	4.19 *	2.5	2.2	0.502 NS
P. aeroginosa		9.10	19.30	5.41 *	1.945	2.9	0.703 *
E. cloacae		0	0	0.00 NS	1.907	0.893	0.786 *
P. mirabilis		0	14.40	4.39 **	2.02	1.82	0.511 NS
P. vulgaris		0	0	0.00 NS	2.8	2.4	0.439 NS
K pneumoniae		0	0	0.00 NS	2.1	2.3	0.378 NS

* (P≤0.05), ** (P≤0.01).

DISCUSSION

Honey is a rich source of carbohydrates, which represent an essential nutrient for bacteria, as they constitute about 95% of its components, in addition to water, antioxidants and other substances (Hegazi *et al.* 2022). Because of the urgent need to obtain alternatives to antibiotics created by the problem of resistance, the topic of bacteriocins had the importance of being one of the solutions to the problem of resistance. The current study used the sidr honey to increase the production of BLIS, and the results revealed that the addition of honey to the culture medium by 1% led to an increase in the diameter of inhibition against bacteria in all producer bacterial isolates and stimulated the production of BLIS in *P. mirabilis*. It is also known that the production of bacteriocin is considered

a competitive characteristic of bacteria produced in the environment. However, the availability of nutrients affects the production of bacteriocin even in the absence of competing species.

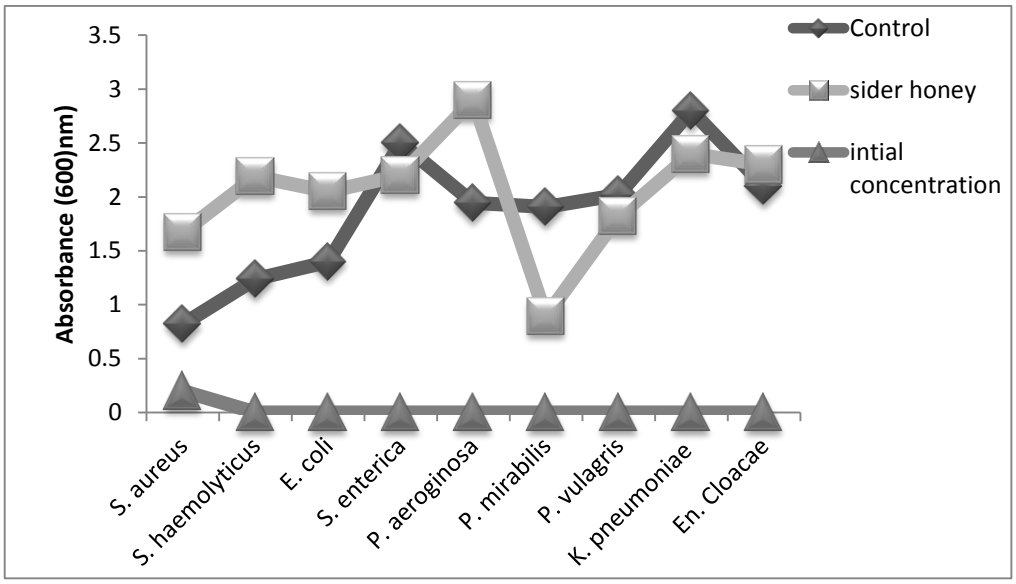

Fig .1. The optical density of bacterial growth in culture medium with and without 1% sidr honey.

This is probably because of the cost of producing bacteriocin compared to the low-nutrient environment, which agrees with (Maldonado-Barragán *et al.* 2013).Microorganisms have effective regulatory system that respond to alterations in the culture medium or environment(Wu *et al.* 2020). Despite the effect of honey inhibiting the growth of bacteria at concentrations higher than 50%(Al-Hasani, 2018), the results showed that adding 1% Sidr honey to the culture medium had a positive effect in increasing the bacterial density in S. *aureus, S. haemolyicus, P. aeroginosa, E. coli, and En. cloacae* except for *P. mirabilis, K. pneumoniae, and P. vulgaris,* compared to when using the culture medium without honey. The results of increased density agree with previous findings, where adding a carbon source to the culture media led to an increase in the density of bacteria(Al-Taie, Al-Musawi & Rasheed 2022). Many reports indicated that the produced BLIS was primary metabolites and and growth associated(Taheri *et al.* 2012).The medium compositions, such as carbon source and minerals, influence the Bacterial growth and production of metabolites (Lee, Kim & Kim 2012). Despite the increased production of BLIS in *P. mirabilis,* the optical density decreased, these species may be affected by the abundance of nutrients, which may reduce their growth rate or be one of the causes of stress, which increases the production of virulence factors. However, no clear explanation exists, so a genetic, environmental, and physiological study is necessary to comprehend this mechanism. In conclusion, the addition of sidr honey affects the production of BLIS in bacteria. It increases the growth rate, and this opens new horizons to study the effect of adding honey on bacterial physiology and gene expression of virulence factors and its relationship to bacterial density.

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Ethics approval
The article does not include any animal experiments or human studies. The ethical approval committees of the College of Medicine/ Al-Iraqia University and the Iraqi Ministry of Health permit the study.

Conflict of interest
There is no conflict of interest

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